Molecular and Cellular Cardiology

Identification of Defects in the Fibrillin Gene and Protein

in Individuals with the Marfan Syndrome and Related Disorders

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The Marfan syndrome is an autosomal dominant disorder with pleiotropic manifestations that involve the cardiovascular, ocular, and skeletal systems. Through a number of investigational approaches, the gene encoding for fibrillin, the FBN1 gene on chromosome 15, has been identified as the defective gene causing the Marfan syndrome. Fibrillin is the large glycoprotein with a repetitive domain structure and is a major protein component of microfibrils, a fibrillar system closely associated with elastin in connective tissue. Mutational analysis of defects in the FBN1 gene in patients with the Marfan syndrome has revealed that most mutations are private or unique in an affected individual or family. Analysis of fibrillin protein or gene defects in individuals with related phenotypes has revealed that a perinatal lethal syndrome, termed neonatal Marfan syndrome, is due to FBN1 gene mutations. In addition, fibroblast cell strains from a subset of patients with idiopathic scoliosis have fibrillin protein defects. Last, fibroblasts from calves affected with bovine Marfan syndrome display defects in the fibrillin protein. These studies have wide-ranging implications in the diagnosis, treatment, and prevention of Marfan syndrome and related disorders. ***(Texas Heart Institute Journal 1994;21:22-9)***

Issue editors:
Ward Casscells, MD
Robert Roberts, MD
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Key words: *Elastic tissue/pathology; fibrillin; Marfan syndrome/genetics; microfibrils*

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This work is supported by a Pfizer Scholars Award to Dr. Milewicz, a Basil O'Conner Award from the March of Dimes, an American Heart Association Grant-In-Aid (93014330), and the National Marfan Foundation.

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The Marfan syndrome is an autosomal dominant disorder with cardiovascular, skeletal, and ocular complications. The disorder has an estimated prevalence of 1 per 10,000 population.[1] Approximately one quarter of patients are born to parents who are not clinically affected, and are thought to result from new mutations in the parental germline. The Marfan syndrome shows high penetrance, and variability in the clinical expression of the disease is common, both between and within families.

The life-threatening complications of the Marfan syndrome are related to the cardiovascular complications. Before the advent of cardiovascular surgery to correct these problems, the life expectancy of an affected patient was greatly decreased, with an average age at death of 32 years.[2] The most serious cardiovascular problem is progressive dilatation of the ascending aorta, which can lead to dissection or rupture if not surgically corrected.[3-5] The majority of patients with the Marfan syndrome also have mitral valve abnormalities, most commonly mitral valve prolapse, but also mitral regurgitation.[6,7]

The skeletal features of the disorder are the most outwardly striking characteristics of the syndrome. Affected individuals typically are very tall, with long arms and legs (dolichostenomelia) and long, thin fingers (arachnodactyly). In addition, they can have skeletal features such as pectus deformities, scoliosis, high-arched palate, pes planus, and joint hypermobility. Cardinal manifestations involving the eye include lens dislocation (ectopia lentis), myopia, and retinal detachment.

In the past few years, intensive work by a number of groups has identified the defective gene causing the Marfan syndrome, the fibrillin or FBN1 gene. Characterizations of mutations in this gene have provided information about the structural domains of the fibrillin protein important for the integrity of the connective tissue. In addition, further work has defined the spectrum of phenotypes associated with FBN1 gene mutations. These studies have wide-ranging implications for the prevention, diagnosis, and treatment of these disorders.

Elastin and Microfibrils

Initial work to elucidate the pathogenesis of the Marfan syndrome involved histopathologic examination of aortas from individuals with the Marfan syndrome. These examinations revealed cystic medial necrosis characterized by focal loss or fragmentation of elastic fibers.[8] Abnormal elastic fibers were observed in other tissues (including lung and skin) from patients with the Marfan syndrome.[9,10] Biochemical analysis of aortic tissue confirmed substantial reduction in elastin content and revealed a 50% decrease in the desmosine (elastin-specific cross-links) content of the isolated elastin.[8] All these results suggested a defect in the formation of elastin fibers. Initially, attention focused on the gene for elastin, the major protein in the elastic fiber. A candidate gene approach was not successful in excluding elastin as the candidate gene, due to a lack of polymorphisms in or around the gene.

The 2 major components of the elastic fiber are an amorphous core of elastin and a matrix of microfibrils that surrounds the amorphous core (Fig. 1). Microfibrils are approximately 10-nm fibers that appear on the periphery of the amorphous core of elastin, but also appear in tissues without elastin.[11,12] Microfibrils appear to play a role in the formation of elastin fibers during development, by providing a scaffold upon which newly synthesized elastin is deposited. The most striking accumulation of microfibrils (without elastin present) is found in the zonule of Zinn, the suspensory ligament of the eye that holds the lens in place. Because microfibrils were found both in the elastic fibers and in the suspensory ligament of the eye, the protein components of the microfibrils became candidates for the defective gene product causing the Marfan syndrome.

In 1986, the major protein component of microfibrils was identified and termed fibrillin.[12] Fibrillin is a large glycoprotein (350 kD) that is found both in microfibrils associated with elastin and in microfibrils not associated with elastin. The protein is found in a wide variety of tissues, including those of the skin, lung, kidney, vessels, cartilage, tendon, muscle, cornea, and suspensory ligament of the eye. Once the major protein component of microfibrils had been identified, the gene that encodes for fibrillin became a major candidate for the defective gene causing the Marfan syndrome.

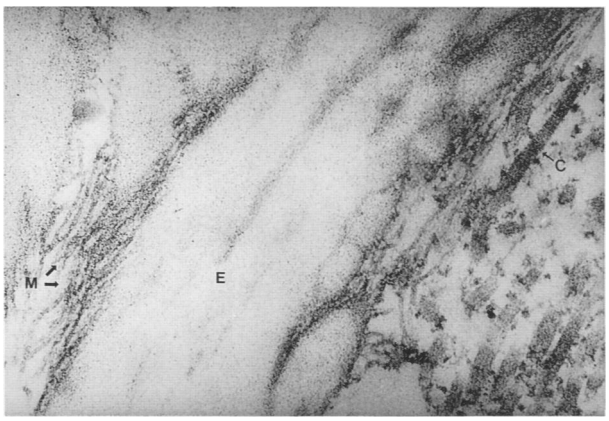

Fig. 1 *Electron microscopic examination of microfibrils (M) around the amorphous core of an elastic fiber (E) in human skin. Larger collagen fibers (C) can be seen.*

Identification of the Genetic Defect

In the late 1980s a number of approaches were used to identify the genetic defect causing the Marfan syndrome (Fig. 2). An international consortium of investigators was carrying out genetic linkage analysis with anonymous polymorphic probes to map the chromosomal locus for the genetic defect causing the Marfan syndrome. Other investigators endeavored to clone, characterize, and determine the chromosomal location of the fibrillin gene. Last, biochemical studies were under way to discover specific defects in the structure, synthesis, and processing of fibrillin by cells from Marfan patients.

The 1st biochemical data to support fibrillin as the defective gene product causing the Marfan syndrome came from work by the late D. Hollister and colleagues.[13-15] Their work strengthened the hypothesis that fibrillin was the defective gene causing the Marfan syndrome by demonstrating decreased immunofluorescence of anti-fibrillin antibody-stained dermal biopsies and cultured fibroblasts from Marfan syndrome patients. Further biochemical evidence that fibrillin was the defective gene product came from our studies of the synthesis, secretion, and processing of fibrillin by dermal fibroblasts from Marfan patients. These studies showed 3 metabolic defects in fibrillin processing by dermal fibroblasts from Marfan patients: decreased fibrillin synthesized by the cell, inefficient secretion of fibrillin, and de-

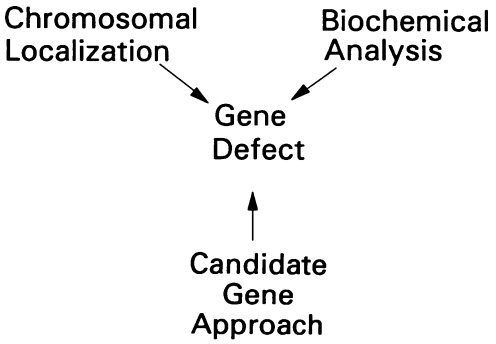

Fig. 2 *Three scientific approaches pursued by different investigators to identify the genetic defect causing the Marfan syndrome.*

creased incorporation of fibrillin into the pericellular matrix, presumably in the form of microfibrils (Fig. 3).[16,17]

By 1990, researchers participating in the international consortium reported exclusion of 75% of the human genome as the site of the Marfan syndrome locus.[18] In 1991, Finnish researchers reported linkage of the Marfan syndrome to the long arm of chromosome 15, 15q15.23.[19] Subsequent studies further defined the locus and showed that all Marfan families studied were linked to this region, i.e., there was no evidence of genetic heterogeneity.[20,21]

The final steps in determining the defective gene causing the Marfan syndrome came with the cloning of the partial cDNA of fibrillin by 2 groups.[22,23] Polymorphic markers within the gene were shown to be closely linked to the Marfan syndrome without recombination.[22,24] In addition, the gene was mapped to 15q15.21, the same chromosomal location to which Marfan syndrome had been mapped using random genomic probes.[22] The final proof came with the identification of a de novo mutation causing the disease in 2 patients with sporadic disease.[24]

The Fibrillin Gene and Protein Structure. The complete cDNA sequence encoding fibrillin was reported in 1993 by 2 research groups.[25,26] The cDNA sequence predicts a cysteine-rich glycoprotein with a repetitive domain structure, as shown in Fig. 4. The fibrillin gene is relatively large (approximately 110 kb). The cDNA is encoded in 64 exons, with almost every cysteine-rich motif encoded by its own exon.[26]

The protein contains 47 repeats that have homology to epidermal growth factor (EGF), 43 of which are predicted to bind calcium, due to the presence of a calcium-binding motif.[27,28] Each of these repeats contains 6 cysteines that are inferred to form the same disulfide bonds as EGF, i.e., disulfide bonds between the 1st and 3rd, 2nd and 4th, and 5th and 6th cysteines. In addition, the protein has repeats with homology to domains found in the transforming growth factor β1 binding protein (TGFβ1 binding protein).[29] These domains are also cysteine rich, containing 8 cysteines each. Fibrillin has other cysteine-rich domains that are variants of the EGF and TGFβ1 binding protein motifs (hybrid motifs). Last, fibrillin has 3 unique domains: an amino-terminal domain, a carboxy-terminal domain, and a proline-rich domain found towards the amino-terminal end of the protein. The protein has a number of putative sites for *N*-linked glycosylation.

Characterization of Mutations in the Fibrillin Gene. A number of mutations in the fibrillin gene have been identified in patients with the Marfan syndrome (Table I[24,25,30-36]). With the exception of the 1st de novo mutation identified in 2 unrelated affected individuals, all mutations have been private

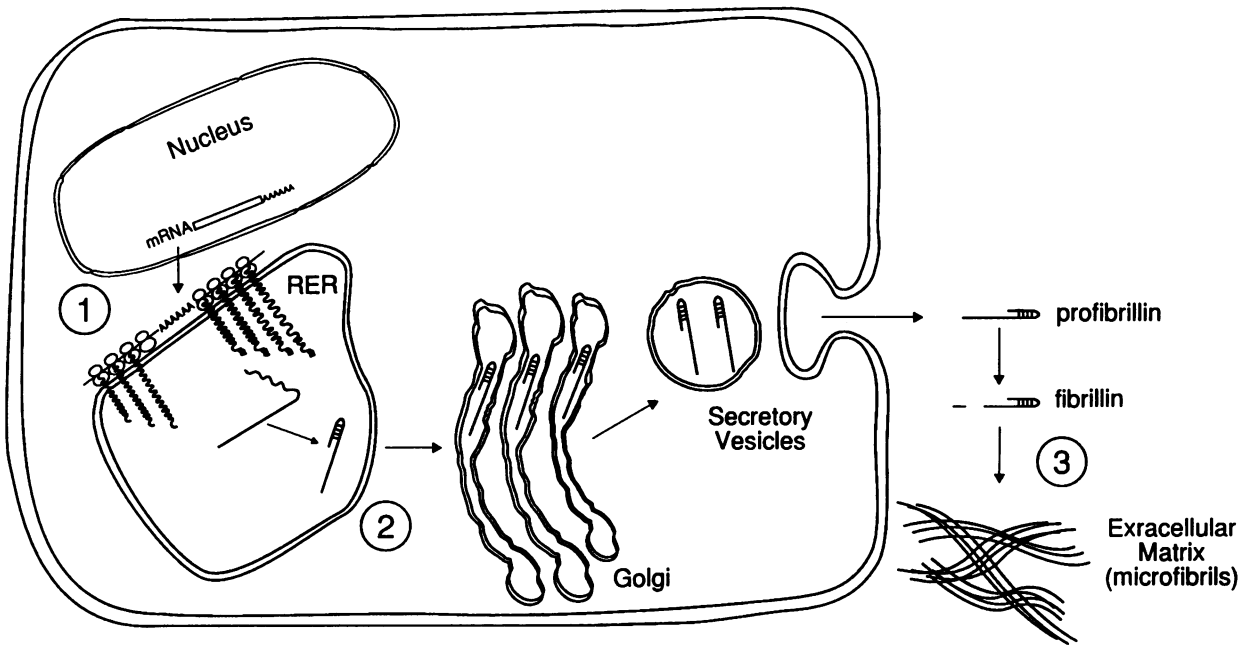

Fig. 3 *Diagrammatic representation of fibrillin synthesis, secretion, proteolytic conversion, and microfibril formation by human dermal fibroblasts. The circled numbers indicate the approximate location of defects in the synthesis (1), secretion (2), and matrix incorporation (3) of fibrillin in dermal fibroblasts from individuals with the Marfan syndrome.*

RER = rough endoplasmic reticulum

(Modified from Milewicz,[17] by copyright permission of the American Society for Clinical Investigation.)

or unique in every family. Currently, there is no correlation between the location of the mutation and the resulting severity of the Marfan syndrome or specific systems affected.

All missense mutations identified are predicted to disrupt EGF-like domains that bind calcium.[24,30-33] Four of the missense mutations involve substitution of 1 of the highly conserved cysteines in the EGF-

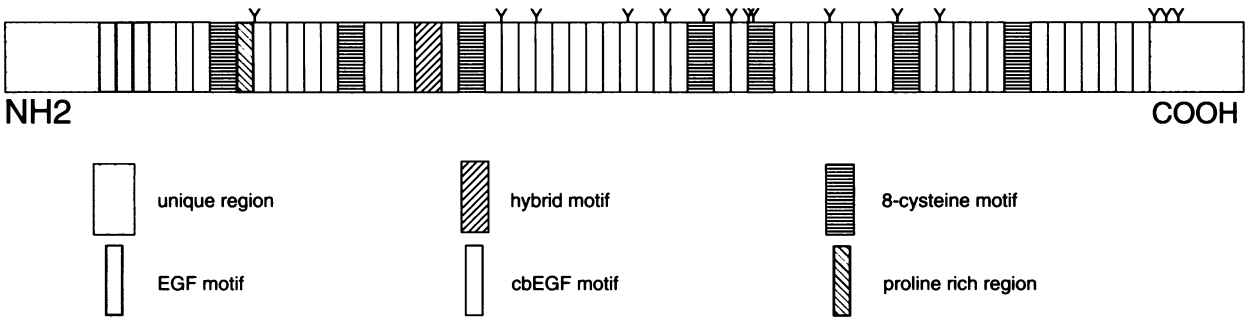

Fig. 4 *Diagrammatic representation of the fibrillin protein structure based on the cDNA sequence. The different domains observed in the fibrillin protein are labeled in the figure legend. The Y characters above the boxes indicate potential sites of N-linked glycosylation based on the amino acid sequence.*

EGF = epidermal growth factor; cbEGF = calcium-binding epidermal growth factor

TABLE I. Identified Mutations in the FBN1 Gene in Marfan Patients

SEQUENCE ALTERATIONS*	AMINO ACID CHANGES*	PREDICTED EFFECT ON PROTEIN
Missense Mutations		
G716C	R239P	Disrupt secondary structure of EGF-like domain[24]
G4226C	C1409S	Disrupt secondary structure of EGF-like domain[30]
G1052C	C351S	Disrupt secondary structure of EGF-like domain[31]
T2293C	C765R	Disrupt secondary structure of EGF-like domain[31]
G3968C	C1323S	Disrupt secondary structure of EGF-like domain[31]
A-1052T	N-351I	Disrupt Ca^{++} binding to EGF-like domain[32]
A-527C	D-176A	Disrupt Ca^{++} binding to EGF-like domain[32]
A3737G	N1246S	Disrupt Ca^{++} binding to EGF-like domain[33]
Nonsense Mutations		
G5574A	W1858X	Premature termination of protein translation[34]
Insertion/Deletions		
2444 ins TTCA	-	Premature termination of protein translation[32]
del -2530 to -2448 (splicing error, G +1 to A)	-	Premature termination of protein translation[32]
del 4762 to 5127 (genomic deletion)	-	Deletion of 3 EGF-like domains with protein translation maintained[34]
del 6664 to 6787 (splicing error, G +1 to C)	-	Deletion of 1 EGF-like domain with protein translation maintained[35]
del 3620 to 3685 (T3645G)	Y1215X	Deletion of 1 EGF-like domain with protein translation maintained[36]

*Nucleotide and amino acid numbering as used by Corson.[25]

like domains, therefore predicted to disrupt the secondary structure of the EGF-like domain by preventing proper disulfide bonding. Specific residues within the EGF-like domains have been shown to be critical for calcium binding.[27,28,37,38] Three missense mutations (N1246S, N-351I, and D-176A) disrupt these amino acids for calcium binding. The last missense mutation (R239P) does not disrupt an amino acid critical for calcium binding, but the substitution of a proline residue for an arginine would disrupt the secondary structure of the domain. Although these missense mutations are predicted to affect the binding of calcium to the EGF-like domain, the role that calcium binding plays in fibrillin function has yet to be elucidated.

Three splicing errors have been identified[32,35,36] as the causative mutation in 3 unrelated Marfan patients. Two of these mutations delete EGF-like domains and leave the reading frame of the protein intact,[35,36] whereas 1 deletes an EGF-like domain and disrupts the reading frame, leading to a truncated protein.[32] Two of these splicing errors are due to mutations at the splice donor site.[32,35] The last splicing error results from a nonsense mutation at position +26 of the deleted exon.[36] Surprisingly, the reading frame of the protein is maintained despite the abnormally spliced exon.

A number of mutations result in the synthesis of a truncated protein. A nonsense mutation in the carboxy terminus is predicted to shorten the protein by 116 amino acids.[34] An insertion of 4 amino acids at nucleotide 2444 leads immediately to a premature stop.[32] Last, a genomic deletion results in deletion of 366-bp from the fibrillin message, producing an in-frame deletion of 122 amino acids.

A number of conclusions can be drawn from the mutational analysis. First, disruption of the secondary structure of EGF-like domains (e.g., disrupting disulfide pairing by mutating 1 of the cysteines) can cause the phenotypic manifestations of the Marfan syndrome. Second, mutations that disrupt the calcium-binding motif in the EGF-like domains can produce the disease. Third, selective deletion of 1 of these domains can also result in the disorder. Last, truncation of the protein, even within the unique carboxy-terminal domain, can produce disease. Surprisingly, no large genomic rearrangements leading to a nonexpressed or null allele have been identified, raising the possibility that a true null allele does not cause typical Marfan syndrome.

Spectrum of Phenotypes Associated with Fibrillin Protein Defects

The varied clinical features of the Marfan syndrome have led to speculation that isolated features of this disorder (or related syndromes) may be due to mutations in the FBN1 gene. One Marfan-related disorder has been shown to be due to mutations in the fibrillin gene, dominantly inherited ectopia lentis.[39] Initial studies of families with dominantly inherited mitral valve prolapse or annuloaortic ectasia have not shown linkage to the FBN1 gene.

We have been interested in investigating the role of fibrillin in a number of clinical disease states. Screening the greater than 8 kb of the fibrillin cDNA for mutations that cause the Marfan syndrome presents a formidable task. Instead, we have relied on biochemical assessment of fibrillin synthesis and processing by using dermal fibroblasts from affected individuals as a screen for possible FBN1 mutations (see Fig. 3). If a protein defect is identified, the fibrillin cDNA is analyzed for a mutation. Using this protocol, we have assessed the role of FBN1 mutations in the following disorders: neonatal Marfan syndrome, adolescent idiopathic scoliosis, and an animal model of the Marfan syndrome. In addition, we are in the process of investigating the role of fibrillin in the pathogenesis of isolated thoracic aortic aneurysms and a number of other disorders.

Neonatal Marfan Syndrome. This is a sporadic, perinatal, lethal syndrome with features of the Marfan syndrome along with manifestations not observed in classic Marfan syndrome.[40-44] Affected children are born with severe cardiovascular problems, including mitral valve regurgitation and progressive aortic root dilatation, and with ocular and skeletal features typical of the Marfan syndrome (Fig. 5). In addition, these patients have congenital contractures, atypical facies, loose, redundant skin, and pulmonary emphysema. These children usually die within the 1st year of life. This more severe and complex phenotype, exhibiting features not typically found in Marfan patients, leads to questions about the molecular defect causing neonatal Marfan syndrome. In our study of an infant with this syndrome, we explanted dermal fibroblasts and found that these cells secreted fibrillin inefficiently, suggesting a mutation in the FBN1 gene. The fibrillin cDNA from the patient's fibroblasts was screened for a mutation and a unique 3-bp insertion was identified between bases 480 and 481 or 481 and 482 of the fibrillin cDNA.[45,46] The insertion maintains the reading frame of the fibrillin protein but inserts an additional cysteine into 1 of the EGF-like repeats. Therefore, we concluded that neonatal Marfan syndrome was due to mutations in the FBN1 gene. We now are investigating why a mutation in this region of the fibrillin protein results in such severe disease.

Adolescent Idiopathic Scoliosis. Scoliosis is 1 of the pleiotropic manifestations of the Marfan syndrome, raising the possibility that individuals with isolated adolescent idiopathic scoliosis may have defects in fibrillin. To answer this question, we have collaborated with Nancy Hadley-Miller, Baylor College of

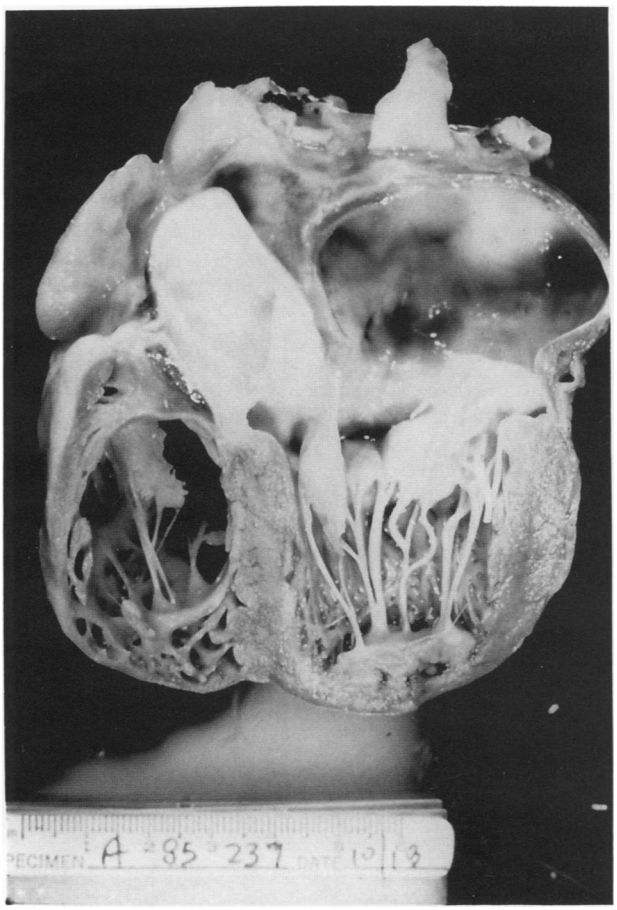

Fig. 5 *Heart at autopsy of a patient with neonatal Marfan syndrome who died at the age of 17 months. Note the enlarged left atrium, ventricles, and aortic root, and the redundant chordae and leaflets of the mitral valve.*

Medicine, to study fibrillin processing by fibroblasts explanted from the ligamentum flavum of individuals with idiopathic scoliosis. We identified a small subset of these patients (4 out of 23, or 17%) who have a defect in fibrillin processing. The specific defect identified in all patients was failure of fibrillin to incorporate into the pericellular matrix surrounding the cells.[47] Fibrillin cDNA from the cell lines that demonstrate a fibrillin defect is now being screened for a FBN1 mutation. The preliminary data suggest that fibrillin defects have a role in the causation of idiopathic scoliosis.

Bovine Marfan Syndrome. The 1st potential animal model of the Marfan syndrome was recently identified by veterinarians at Washington State University.[48] Bovine Marfan syndrome resembles human Marfan syndrome in that affected cows have skeletal anomalies (dolichostenomelia, joint hypermobility), ocular findings (ectopia lentis), and cardiovascular lesions (aortic root dilatation and rupture) that are similar to features observed in human beings with Marfan syndrome (Fig. 6). To determine if fibrillin processing was abnormal, we obtained dermal fibroblasts from affected calves and age-matched controls.[49] Metabolic labeling studies of fibrillin showed clearly that cells from affected calves did not incorporate fibrillin into the pericellular matrix, whereas cells from normal calves demonstrated normal incorporation of fibrillin. In conclusion, these results suggest that the bovine Marfan syndrome is also caused by a mutation in fibrillin, leading to defective incorporation into microfibrils.

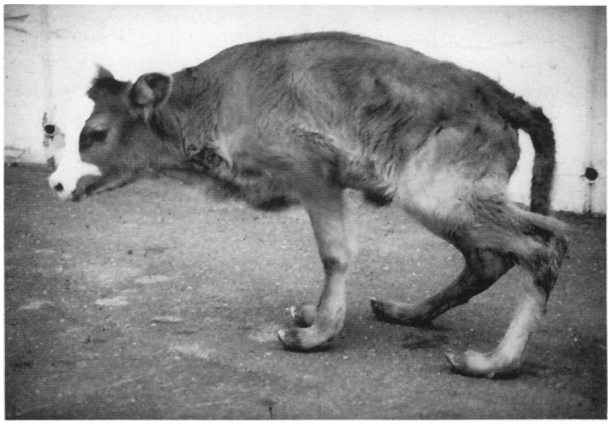

Fig. 6 *Bovine Marfan syndrome. This calf was diagnosed as affected based upon hypermobility of distal limbs, dolichostenomelia, ectopia lentis, and aortic root dilatation.*

Conclusion

Research over the past 4 years has identified the FBN1 or fibrillin gene as the defective gene causing the Marfan syndrome. Characterization of mutations in the FBN1 gene in individuals with the disorder has provided some preliminary information about the functional significance of some regions of the fibrillin protein, but further work is needed to complete our understanding of how fibrillin defects cause the Marfan syndrome. Last, work is in progress to determine the full range of clinical disease associated with FBN1 mutations.

References

1. Pyeritz RE. The Marfan syndrome. In: Royce PM, Steinmann B, eds. Connective tissue and its heritable disorders. New York: Wiley-Liss and Sons, 1993:437-68.
2. Murdoch JL, Walker BA, Halpern BL, Kuzma JW, McKusick VA. Life expectancy and causes of death in the Marfan syndrome. N Engl J Med 1972;286:804-8.
3. Marsalese DL, Moodie DS, Vacante M, et al. Marfan's syndrome: natural history and long-term follow-up of cardiovascular involvement. J Am Coll Cardiol 1989;14:422-31.
4. Child JS, Perloff JK, Kaplan S. The heart of the matter: cardiovascular involvement in Marfan's syndrome. J Am Coll Cardiol 1989;14:429-31.
5. Roberts WC, Honig HS. The spectrum of cardiovascular disease in the Marfan syndrome: a clinico-morphologic study

of 18 necropsy patients and comparison to 151 previously reported necropsy patients. Am Heart J 1982;104:115-35.
6. Pini R, Roman MJ, Kramer-Fox R, Devereux RB. Mitral valve dimensions and motion in Marfan patients with and without mitral valve prolapse. Comparison to primary mitral valve prolapse and normal subjects. Circulation 1989;80:915-24.
7. Pyeritz RE, Wappel MA. Mitral valve dysfunction in the Marfan syndrome: clinical and echocardiographic study of prevalence and natural history. Am J Med 1983;74:797-807.
8. Perejda AJ, Abraham PA, Carnes WH, Coulson WF, Uitto J. Marfan's syndrome: structural, biochemical, and mechanical studies of the aortic media. J Lab Clin Med 1985;106:376-83.
9. Holbrook KA, Byers PH. Structural abnormalities in the dermal collagen and elastic matrix from the skin of patients with inherited connective tissue disorders. J Invest Dermatol 1982;79(Suppl 1):7s-16s.
10. Sayers CP, Goltz RW, Mottaz J. Pulmonary elastic tissue in generalized elastolysis (cutis laxa) and Marfan's syndrome. A light and electron microscopic study. J Invest Dermatol 1975;65:451-7.
11. Goldfischer S, Coltoff-Schiller B, Goldfischer M. Microfibrils, elastic anchoring components of the extracellular matrix, are associated with fibronectin in the zonule of Zinn and aorta. Tissue Cell 1985;17:441-50.
12. Sakai LY, Keene DR, Engvall E. Fibrillin, a new 350-kD glycoprotein, is a component of extracellular microfibrils. J Cell Biol 1986;103:2499-509.
13. Hollister DW, Godfrey M, Sakai LY, Pyeritz RE. Immunohistologic abnormalities of the microfibrillar-fiber system in the Marfan syndrome. N Engl J Med 1990;323:152-9.
14. Godfrey M, Olson S, Burgio RG, et al. Unilateral microfibrillar abnormalities in a case of asymmetric Marfan syndrome. Am J Hum Genet 1990;46:661-71.
15. Godfrey M, Menashe V, Weleber RG, et al. Cosegregation of elastin-associated microfibrillar abnormalities with the Marfan phenotype in families. Am J Hum Genet 1990;46: 652-60.
16. McGookey DJ, Smith ACM, Waldstein G, Byers PH. Mosaicism for a deletion in one of the type III collagen alleles indicates that the deletion occurred after identification of cells for recruitment to different cell lineages early in human development [abstract]. Am J Hum Genet 1989;45:809.
17. Milewicz DM, Pyeritz RE, Crawford ES, Byers PH. Marfan syndrome: defective synthesis, secretion, and extracellular matrix formation of fibrillin by cultured dermal fibroblasts. J Clin Invest 1992;89:79-86.
18. Blanton SH, Sarfarazi M, Eiberg H, et al. An exclusion map of Marfan syndrome. J Med Genet 1990;27:73-7.
19. Kainulainen K, Pulkkinen L, Savolainen A, Kaitila I, Peltonen L. Location on chromosome 15 of the gene defect causing Marfan syndrome. N Engl J Med 1990;323:935-9.
20. Kainulainen K, Steinmann B, Collins F, et al. Marfan syndrome: no evidence for heterogeneity in different populations, and more precise mapping of the gene. Am J Hum Genet 1991;49:662-7.
21. Dietz HC, Pyeritz RE, Hall BD, et al. The Marfan syndrome locus: confirmation of assignment to chromosome 15 and identification of tightly linked markers at 15q15-q21.3. Genomics 1991;9:355-61.
22. Lee B, Godfrey M, Vitale E, et al. Linkage of Marfan syndrome and a phenotypically related disorder to two different fibrillin genes. Nature 1991;352(6333):330-4.
23. Maslen CL, Corson GM, Maddox BK, Glanville RW, Sakai LY. Partial sequence of a candidate gene for the Marfan syndrome. Nature 1991;352(6333):334-7.
24. Dietz HC, Cutting GR, Pyeritz RE, at al. Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene. Nature 1991;352(6333):337-9.
25. Corson GM, Chalberg SC, Dietz HC, Charbonneau NL, Sakai LY. Fibrillin binds calcium and is coded by cDNAs that reveal a multidomain structure and alternatively spliced exons at the 5′end. Genomics 1993;17:476-84.
26. Pereira L, D'Alessio M, Ramirez F, et al. Genomic organization of the sequence coding for fibrillin, the defective gene product in Marfan syndrome. Hum Mol Genet 1993;2:961-8.
27. Mayhew M, Handford P, Baron M, Tse AG, Campbell ID, Brownlee GG. Ligand requirements for Ca^{2+} binding to EGF-like domains. Protein Eng 1992;5:489-94.
28. Selander-Sunnerhagen M, Ullner M, Persson E, Teleman O, Stenflo J, Drakenberg T. How an epidermal growth factor (EGF)-like domain binds calcium. High resolution NMR structure of the calcium form of the NH2-terminal EGF-like domain in coagulation factor X. J Biol Chem 1992;267: 19642-9.
29. Kanzaki T, Olofsson A, Moren A, et al. TGF-beta 1 binding protein: a component of the large latent complex of TGF-beta 1 with multiple repeat sequences. Cell 1990;61:1051-61.
30. Dietz HC, Pyeritz RE, Puffenberger EG, et al. Marfan phenotype variability in a family segregating a missense mutation in the epidermal growth factor-like motif of the fibrillin gene. J Clin Invest 1992;89:1674-80.
31. Dietz HC, Saraiva JM, Pyeritz RE, Cutting GR, Francomano CA. Clustering of fibrillin (FBN1) missense mutations in Marfan syndrome patients at cysteine residues in EGF-like domains. Hum Mutat 1992;1:366-74.
32. Dietz HC, McIntosh I, Sakai LY, at al. Four novel FBN1 mutations: significance for mutant transcript level and EGF-like domain calcium binding in the pathogenesis of Marfan syndrome. Genomics 1993;17:468-75.
33. Hewett DR, Lynch JR, Smith R, Sykes BC. A novel fibrillin mutation in the Marfan syndrome which could disrupt calcium binding of the epidermal growth factor-like module. Hum Mol Genet 1993;2:475-7.
34. Kainulainen K, Sakai LY, Child A, et al. Two mutations in Marfan syndrome resulting in truncated fibrillin polypeptides. Proc Natl Acad Sci U S A 1992;89:5917-21.
35. Godfrey M, Vandemark N, Wang M, et al. Prenatal diagnosis and a donor splice site mutation in fibrillin in a family with Marfan syndrome. Am J Hum Genet 1993;53:472-80.
36. Dietz HC, Valle D, Francomano CA, Kendzior RJ Jr, Pyeritz RE, Cutting GR. The skipping of constitutive exons in vivo induced by nonsense mutations. Science 1993;259:680-3.
37. Handford PA, Mayhew M, Baron M, Winship PR, Campbell ID, Brownlee GG. Key residues involved in calcium-binding motifs in EGF-like domains. Nature 1991;351(6322): 164-7.
38. Handford PA, Baron M, Mayhew M, et al. The first EGF-like domain from human factor IX contains a high-affinity calcium binding site. EMBO J 1990;9:475-80.
39. Tsipouras P, Del Mastro R, Sarfarazi M, et al. Genetic linkage of the Marfan syndrome, ectopia lentis, and congenital contractural arachnodactyly to the fibrillin genes on chromosomes 15 and 5. The International Marfan Syndrome Collaborative Study. N Engl J Med 1992;326:905-9.
40. Gross DM, Robinson LK, Smith LT, Glass N, Rosenberg H, Duvic M. Severe perinatal Marfan syndrome. Pediatrics 1989; 84:83-9.
41. Shankar KR, Hultgren MK, Lauer RM, Diehl AM. Lethal tricuspid and mitral regurgitation in Marfan's syndrome. Am J Cardiol 1967;20:122-8.
42. Lababidi Z, Monzon C. Early cardiac manifestations of Marfan's syndrome in the newborn. Am Heart j 1981;102: 943-5.
43. Day DL, Burke BA. Pulmonary emphysema in a neonate with Marfan syndrome. Pediatr Radiol 1986;16:518-21.

44. Huggon IC, Burke JP, Talbot JF. Contractural arachnodactyly with mitral regurgitation and iridodonesis. Arch Dis Child 1990;65:317-9.
45. Milewicz DM, Duvic MD. Severe neonatal Marfan syndrome resulting from de novo three base pair insertion into the fibrillin gene on chromosome 15. Am J Hum Genet [in press].
46. Milewicz DM, Duvic M. Three base pair insertion into fibrillin 15 gene causes severe perinatal Marfan syndrome [abstract]. Clin Res 1993;41:259a.
47. Hadley-Miller N, Mims B, Milewicz DM. Demonstration of elastic fiber pathology in patients with adolescent idiopathic scoliosis. J Bone Joint Surg [in press].
48. Besser TE, Potter KA, Bryan GM, Knowlen GG. An animal model of the Marfan syndrome. Am J Med Genet 1990;37: 159-65.
49. Potter KA, Hoffman Y, Sakai LY, Byers PH, Besser TE, Milewicz DM. Abnormal fibrillin metabolism in bovine Marfan syndrome. Am J Pathol 1993;142:803-10.